Journal of Gerontology: BIOLOGICAL SCIENCES
1990, Vol. 45, No. 5, B148–163

Dietary Restriction and Aging: The Initiation of a Primate Study

Donald K. Ingram,[1] Richard G. Cutler,[1] Richard Weindruch,[2] David M. Renquist,[3] Joseph J. Knapka,[3] Milton April,[3] Claude T. Belcher,[3] Margaret A. Clark,[3] Charles D. Hatcherson,[3] Bernadette M. Marriott,[4] and George S. Roth[1]

[1]Molecular Physiology and Genetics Section, Gerontology Research Center, NIA, NIH, Baltimore, MD.
[2]Biomedical Research and Clinical Medicine Program, NIA, NIH.
[3]Veterinary Resources Branch, Division of Research Services, NIH.
[4]Department of Comparative Medicine, Johns Hopkins University.

Juvenile (1 yr) and adult (3-5 yr) male rhesus monkeys* (Macaca mulatta) *andjuvenile (1–4 yr) and adult (S-10 yr) male squirrel monkeys* (Saimiri sciureus) *were fed a diet at or near ad libitum levels based on recommended caloric intake for age and body weight* or *fed 30% less of the same diet with this restriction gradually introduced over a 3-mo period. Analysis of body weights among these respective control and experimental groups from the first year of the study indicated that the monkeys undergoing dietary restriction were gaining weight at a markedly slower rate compared to control values. Actual food intake among diet-restricted groups had been reduced 22–24% below control levels. Periodic analysis of hematology and blood chemistry measurements over the first year of the study detected few significant differences between control and experimental groups to indicate that diet restriction was not detrimental to general health. When values obtained from hematology and blood chemistry measurements of juvenile and adult groups (control and experimental groups combined) were compared to ad libitum fed old monkeys from each species (> 18 yr for rhesus; > 10 yr for squirrel monkeys), many significant age differences were noted. Among the largest and most consistent findings in both species were age-related decreases in concentrations of lymphocytes, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, and phosphates as well as the albuminlglobulin ratio and the blood urea nitrogenlcreatinine ratio. Age-related increases in serum globulin and creatinine concentrations were also found. These parameters as well as many others being implemented in the study will be monitoredfurther to determine if diet restriction affects the rate of development as well as aging as observed in numerous rodent studies applying such nutritional manipulations.

OVER five decades ago it was first recognized that the life span of laboratory rats could be increased up to 50% relative to ad libitum fed controls by dietary restriction (McCay et al., 1935). This observation has been repeated in numerous species, such as flies, water fleas, fish, and mice with various feeding regimens (for reviews see Barrows and Kokkonen, 1984; Weindruch and Walford, 1988). In rats and mice, the increase in maximum life span appears to depend primarily on caloric restriction, which is associated with later appearances and lower incidences of most spontaneous diseases and retarded rates of change for most age-sensitive parameters studied to date (Weindruch and Walford, 1988).

The experimental evidence that dietary restriction lengthens life span of different species suggests similar effects may be expected in human and nonhuman primate species. However, it is important to emphasize that all previous dietary restriction experiments have utilized species having considerably shorter life spans than primates. Since little is known about mechanisms governing species differences in longevity or how dietary restriction increases life span, it is possible that, as the natural (ad libitum-fed) life span of different species increases, the effect of dietary restriction on life span extension would decrease (Cutler, 1984). This prediction is based on the possibility that life extension mechanisms induced by dietary restriction of short-lived species, such as rats or mice, may be normally expressed in species that are naturally longer-lived, even under ad libitum conditions. If this hypothesis were true, then the life span extension effect of dietary restriction in primates should be considerably less than that observed in shorter-lived rodent species. Regarding diet and aging, it is difficult to make comparisons between humans and other mammals because animals do not always eat ad libitum in the wild and old individuals are rarely found due to increased susceptibility to predators. Thus, the possibility of dietary restriction affecting human aging is controversial.

In light of the intense recent interest in the mechanisms, physiological validity, and possible applicability of dietary restriction to humans (Masoro, 1988; Walford, 1986), it has become essential that this phenomenon be tested in animal models more closely related to humans. We have chosen two primate species, *Macaca mulatta* (rhesus monkey) and *Saimiri sciurius* (squirrel monkey), representing Old and New World monkeys, respectively, with which to examine the effects of dietary restriction on aging processes. The twofold difference in maximal life spans of these species, which are approximately 40 and 20 years, respectively (Jones, 1968), provides an opportunity to assess and to compare possible dietary-induced alterations on aging. We are evaluating a battery of noninvasive physiological and biochemical tests that might index aging processes in various

systems. These have been selected to monitor health status as well as to correlate with chronological age in primates or other species (Kessler et al., 1983; Short et al., 1987; Weindruch and Walford, 1988).

The dietary restriction regimen involved a gradually imposed 30% reduction in total food intake from estimated ad libitum levels. This objective was selected on the basis of rodent studies to affect aging processes without severely retarding growth or impairing health. To guard against malnutrition, the diet has been supplemented with vitamins and minerals to provide all animals at least the minimum recommended allowance. Three age groups of males were studied for each species: juvenile, young adult, and old. All old animals were fed ad libitum and were included to provide cross-sectional age comparisons. Juvenile and young adults were assigned to control and experimental subgroups to coincide with those ages most affected by diet in rodent studies (Weindruch and Walford, 1988). Findings on food intake, body weight, blood chemistries and cell counts from the first year of this project are reported.

METHODS

Animal Procurement

The procurement and quarantine of all animals were supervised by personnel in the Division of Research Services, NIH, at the Primate Unit (Poolesville, MD) using standard procedures. Upon arrival at the Poolesville facility, each monkey was tattooed on the chest for identification and quarantined for 60 days. The monkeys continue to be maintained at Poolesville.

Male rhesus monkeys *(Macaca mulatta)* were acquired during December 1986–February 1987 and consisted of: 12 juveniles (0.61 .O yr, M = 0.9 yr), 12 young adults (3-5 yr, M = 4.2 yr), and 6 old (> 18-25 yr, M = 19.5 yr). Both the juveniles and adults were born in captivity. The juvenile monkeys were born, reared, and obtained from the NIH facility at Perrine, Florida via a short stay at the Primate Research Institute (PRI at the New Mexico State University, Holloman Air Force Base). The adults were obtained from a research colony in the People's Republic of China via the Texas Primate Center (TPC). The old group was captured in India sometime after 6 years of age and was classified as full adult on arrival. They were obtained from breeding colonies at Perrine, where they had been housed in social mixed age and sex breeding groups. The ages of the rhesus monkeys were determined by dental archives and historical receipt records, which were fairly detailed. According to the available records, no monkey had been used in invasive experiments prior to procurement.

Obtained in February 1987, the squirrel monkeys *(Saimiri sciureus)* were males grouped by age as follows: 12 juvenile (l-4 yr), 13 adult (5-10 yr), and4 old (> 10 yr). Most were captured from the wild and purchased from World Wide Primates (Dallas, TX). Virtually all monkeys were identified by morphometric characteristics and selected cytogenetic typing on some animals to be of the *sciureus* subspecies, except for 6 monkeys (2 adult and 4 old obtain.ed from the Primate facility at Iquitos, Peru) that were typed as the *boliviensis* subspecies. An additional subject *(sciureus* subspecies) was obtained from the Naval Medical Research Center to replace one of the adult monkeys that died shortly after arrival. As with the rhesus subjects, all squirrel monkeys had been free from invasive experimentation.

Housing

The monkeys in this study were under the care of staff veterinarians (M.A., D.M.R.) and trained primate technicians (C.T. B., M.A.C., C.D.H.) and maintained using the Primate Unit's standard procedures with the modifications described below.

Except for the aged rhesus monkeys, which were individually caged because of a greater propensity for fighting, all animals were housed originally in pairs with cagemates of matching species, diet, and approximate age as estimated at the outset of the study. Pair housing of monkeys was used to strike a balance between social needs of the animals and experimental needs of controlling food intake. Two or three interconnected stainless steel cages (88.9 cm high by 61 .O cm wide by 68.5 cm deep each) were used per monkey pair. The triple cages were used for all juvenile and adult pairs of rhesus monkeys. Double cages were used for selected large individuals from the old rhesus group. The squirrel monkey pairs were all housed in double-connected cages. The interconnected cages had a sliding partition to isolate cagemates during feeding and thus to control individual food intake. A wire screen (0.6 cm) placed approximately 8 cm below the cages enabled monkeys to retrieve dropped food and facilitated measurement of individual food intake.

The rooms, 2.9 x 8.2 m in size, contained no windows but had temperature (22-28 °C), humidity (50–60%) and light-cycle (12 hr on; 12 hr off) under automatic control. All light was fluorescent, providing 30 ft candles of illumination per room. Ventilation was 100% fresh air at 10-l 5 changes per hour with standard filtration. Water was provided to each cage ad libitum via an automatic, filtered, and chlorinated (2-3 ppm) watering system. Each cage contained a white Teflon ball (about 5.1 cm diameter) that has been used as an environmental stimulant for laboratory primates (Renquist and Judge, 1985). Cages were washed twice daily and removed for steam cleaning on a regular basis.

Health monitoring. — All animals were observed daily. Physical examinations and hematologic studies were conducted in each monkey every 3 months. Fecal study for parasites was analyzed in samples from randomly selected monkeys every 3 months. Routine tuberculin testing and dental cleaning were performed twice yearly.

Experimental Design

Five subgroups of rhesus monkeys were established using juvenile (0.6-l yr), young adult (3-5 yr), and old (18–25 yr) animals: (1) a juvenile control group fed the diet at an approximate ad libitum level based on estimated energy requirements for their age and weight (n = 6); (2) a juvenile experimental group subjected to 30% dietary restriction relative to control values (n = 6); (3) an adult control group (n = 6); (4) an adult experimental group (n = 6); and (5) an old control group (n = 6).

Five subgroups of squirrel monkeys were also established

using juvenile (1–4 yr), adult (5-9 yr), and old (> 10 yr) animals as follows: (1) juvenile control (n = 7); (2) juvenile experimental (n = 5); (3) adult control (n = 7); (4) adult experimental (n = 6); and (5) old control (n = 4).

Within age groups of both species, cagemates and diet groups were matched on the basis of body weight. Where records indicated the inclusion of siblings or half-siblings, these were balanced across control and experimental groups to the extent possible. Because only broad age range estimates were originally available for the wild-caught squirrel monkeys, revised age estimations were based on morphometric and dental parameters made after the initial groupings. Accordingly, several individuals were reclassified by age groups after the study had been initiated.

Diets and Feeding Strategies

Separate natural ingredient diets were used for each species. Each diet was formulated by an experienced primate nutritionist (J.J.K.) to contain the required concentration of nutrients and was manufactured into a palatable form and texture. Nutrient concentrations were based on published estimates of requirements for nonhuman primates (National Research Council, 1978) and on the nutrient concentration in commercially available diets that have resulted in acceptable performance when consumed by these species.

Because the objective of this study was to offer food to experimental animals at 70% of control levels without risking nutrient deficiency, the concentration of vitamin and mineral premixes used in the diets fed to all animals was increased by 40% above the amount considered adequate for ad libitum fed animals. Thus, the diet-restricted monkeys received approximately 100 percent of the recommended daily allowance. Another advantage of this procedure was the elimination of the potential variables that are introduced when different diets are fed to experimental and control groups.

Diet formulations. — The formulation for the diet fed to the rhesus monkeys (Table 1) is a modification of the high-fiber diet routinely fed to monkeys maintained at the National Institutes of Health. The modification involved lowering the crude fiber content from 7 to 5% in an attempt to more closely match the levels found in most commercially available primate diets. The diet was manufactured (Agway, Ithaca, NY) by extruding into biscuits (0.71-O-95 cm thick, 1.59–2.54 cm long).

The formulation for the diet fed to the squirrel monkeys (Table 2) is a modification of the one used for a marmoset and tamarin diet (Barnard et al., 1988). The difference is the lowering of crude fat concentration from about 10 to 8%. This diet was manufactured (Zeigler, Gardners, PA) using cold pelleting techniques into 1.27 cm round pellets, 1.91-3.18 cm in length.

The calculated nutrient concentrations of both diets are presented in Table 3. The diet fed to squirrel monkeys contains a higher content of energy (33% higher), protein (30% higher), and fat (59% higher) than is in the diet fed to rhesus monkeys.

Diet management. — The rhesus diet was manufactured in 1,8 14 Kg batches, the squirrel monkey diet in 454 kg batches, and stored in a freezer (< − 18 °C) until use. Test

Table 1. Formulation for the Rhesus Monkey Diet

Ingredient	Amount (% by Weight)
Basal Mix[a]	
Ground wheat	35.50
Ground corn	22.10
Soybean hulls	12.00
Soybean meal (48% protein)	8.50
Fish meal (60% protein)	5.47
Sugar	4.00
Alfalfa meal	3.00
Dried whey	3.00
Brewers yeast	2.00
Limestone	1.30
Dicalcium phosphate	1.00
Iodized salt	0.60
Mineral mix	0.40
dl-methionine	0.13
Vitamin mix	1.00
	100.00
Complete Diet[b]	
Basal mix	96.75
Soybean oil	3.10
Vitamin C	0.15
	100.00

Mineral	Mineral Premix: Amount (per 100 lb product)	Source
Cobalt	30.0 mg	Cobalt carbonate
Copper	500.0 mg	Copper sulfate
Iron	3.0 g	Iron sulfate
Magnesium	22.0 g	Magnesium oxide
Manganese	3.0 g	Manganous oxide
Potassium	25.0 g	Potassium bicarbonate
Zinc	3.7 g	Zinc oxide
Iodine	100.0 mg	Calcium iodate

Vitamin	Vitamin Premix: Amount (per 100 lb product)	Source
Vitamin A	680.000 IU	Stabilized Vitamin A
Vitamin D	230.000 IU	D-3
Vitamin E	3.0 g	dl-alpha-tocopheryl acetate
Vitamin B_{12}	850.0 mcg	
Riboflavin	350.0 mg	
Niacin	3.0 g	
Pantothenic acid	3.0 g	d-calcium pantothenate
Choline	20.0 g	Choline chloride
Meandione activity	500.0 mg	
Folic acid	400.0 mg	
Thiamin	250.0 mg	Thiamin mononitrate
Pyridoxine	500.0 mg	
Biotin	5.0 mg	d-biotin

[a]The Basal Mix was extruded.

[b]The soybean oil and Vitamin C are sprayed on the outside of the extruded biscuits.

Table 2. Formulation for the Squirrel Monkey Diet

Ingredient	Amount (% by Weight)
Basal Mix	
Fish meal (60% protein)	10.00
Soybean meal (48% protein)	12.00
High fat milk solids	6.50
Corn flour	25.50
Casein	6.40
Glucose	17.90
Apple pomace	10.00
Beet pulp	5.00
Soybean oil	1.90
Soybean lecithin	0.60
Dicalcium phosphate	1.00
Calcium carbonate	0.60
Salt	0.60
Mineral mix	1.00
Vitamin mix	1.00
	100.00

Mineral Premix		
Mineral	Amount (per 100 lb product)	Source
Cobalt	50.0 mg	Cobalt carbonate
Copper	900.0 mg	Copper sulfate
Iron	5.0 g	Iron sulfate
Magnesium	50.0 g	Magnesium oxide
Manganese	43.5 g	Manganous oxide
Potassium	232.0 g	Potassium bicarbonate
Zinc	4.5 g	Zinc oxide
Iodine	100.0 mg	Calcium iodate

Vitamin Premix		
Vitamin	Amount (per 100 lb product)	Source
Vitamin A	680.000 IU	Stabilized Vitamin A
Vitamin D	230,000 IU	D-3
Vitamin E	3.5 g	dl-alpha-tocopheryl acetate
Vitamin B_{12}	1.300 mcg	
Riboflavin	550.0 mg	
Niacin	4.7 g	
Pantothenic acid	3.4 g	d-calcium pantothenate
Choline	65.8 g	Choline chloride
Meandione activity	500.0 mg	
Folic acid	500.0 mg	
Thiamin	500.0 mg	Thiamin mononitrate
Pyridoxine	700.0 mg	
Biotin	15.0 mg	d-biotin
Vitamin C	50.0 g	

Table 3. Calculated Nutrient Concentrations of the Diets

Nutrient	Units	Rhesus Monkey Diet	Squirrel Monkey Diet
Crude protein	%	15.37	20.34
Crude fat	%	5.01	7.97
Crude fiber	%	5.00	4.99
Gross energy	Kcal/g	3.77	4.03
Amino Acids (% of total diet)			
Arginine		.86	1.14
Lysine		.88	1.34
Methionine		.32	.47
Cystine		.24	.21
Tryptophan		.21	.24
Glycine		.91	.90
Histidine		.37	.52
Leucine		1.22	1.75
Isoleucine		.83	1.07
Phenylalanine		.76	.99
Tyrosine		.52	.80
Threonine		.65	.85
Valine		.85	1.15
Minerals			
Calcium	%	1.12	1.13
Phosphorous	%	0.70	0.70
Potassium	%	0.63	0.63
Sodium	%	0.35	0.35
Magnesium	%	0.21	0.22
Iron	mg/kg	301.0	230.0
Zinc	mg/kg	101.0	100.4
Manganese	mg/kg	99.0	99.4
Copper	mg/kg	22.8	23.1
Cobalt	mg/kg	1.0	1.1
Iodine	mg/kg	2.4	2.2
Vitamins			
Vitamin A	IU/g	15.0	15.0
Vitamin D	IU/g	5.0	5.0
Vitamin E	mg/kg	76.8	77.4
Vitamin K	mg/kg	11.0	11.0
Thiamin	mg/kg	10.4	11.0
Riboflavin	mg/kg	12.0	12.1
Niacin	mg/kg	104.0	103.8
Pantothenic acid	mg/kg	74.0	74.8
Choline	mg/kg	1470.0	1470.0
Folic acid	mg/kg	10.1	11.0
Biotin	mg/kg	0.3	0.3
Pyridoxine	mg/kg	14.8	15.4
Vitamin B_{12}	ug/kg	28.7	28.8
Vitamin C	mg/kg	1100.0	1100.0

samples were collected from each production batch of diet prior to freezer storage, and at 6-month intervals during the storage period these were sent to an independent laboratory (Lancaster Laboratory, Lancaster, PA) for nutrient and contaminant assays. The assays indicated that (a) the nutrients were at the expected levels, (b) contaminant concentrations did not exceed the recommended limits for toxicology studies (Rao and Knapka, 1987), and (c) freezer storage had little influence on nutrient c'bncentrations.

Feeding practices. — Potential for error exists in determining the ad libitum food intake of nonhuman primates due to their untidy feeding behaviors in captivity. Often pellets or biscuits are spread throughout the home cage or in areas of the animal room. The nutritional objective for this study was to provide sufficient food for control groups to meet their estimated energy requirements (National Research Council, 1978) based on their age and individual mean body weight over the preceding 3-mo period (Table 4). The experimental

animals were introduced to food restriction during a 3-mo transition period (90% of ad libitum intake during the first month, 80% during the second month, and 70% thereafter).

Food was offered in a stainless steel tray mounted on the outside of each cage. Each animal was moved into an individual cage about 0700 and fed a measured amount (50%) of the daily allotment. The remainder of the daily ration was offered at 1330. At 1500 all unconsumed food was removed, and the amount of uneaten food determined. The monkeys were then caged in their designated pairings until the next morning. During selected intervals, 'uneaten food was weighed after being dried in an oven, and the amount consumed for each monkey determined as a percent of the average dry weight of pellets. Also, each monkey was provided with a small amount of fresh fruit on a weekly schedule.

Experimental Measurements

Monthly, monkeys were anesthetized (7-10 mg/kg ketamine i.m.) prior to the morning feeding. When the monkey was supine, it was removed quickly from the cage, weighed on an electronic balance, and blood samples (12 ml for rhesus, 4 ml for squirrel) were drawn from the femoral vein. Because blood samples were obtained within 10 min of ketamine administration, effects of this anesthetic on hematological and serum biochemical values were considered minimal (Yoshida et al., 1986). Standard hematology and blood chemistry assays were performed at the Maryland Medical Laboratory (Baltimore, MD). These data were thus collected monthly during baseline and the 3-month transition to dietary restriction (5 occasions), and quarterly after that (2 occasions analyzed in current report).

RESULTS

Body Weight

One year of dietary restriction reduced rates of body weight increase in juvenile and adult monkeys of both species relative to respective control groups. Several statistical analyses were applied to confirm this observation and to compare body weight changes among groups.

Individual body weights obtained at monthly weighings reveal considerable heterogeneity (Figure 1). Mean (*SEM*s) monthly body weights for each group (Figures 2-3) illustrate three obvious differences without statistical analysis: (1) the body weights of rhesus monkeys were generally greater (4-10 times higher) compared to squirrel monkeys in comparable age groups; (2) each species showed an age-related increase in body weight; and (3) the effect of diet on body weight is much more apparent in rhesus compared to squirrel monkeys.

At the outset of the study, control and experimental groups were matched on the basis of body weight, such that no statistical difference existed ($p > .05$, according to two-tailed t-tests). The reassignment of ages for some of the squirrel monkeys as mentioned earlier resulted in the appearance of a difference in starting weight (Figure 3) in the juvenile group, but it was not statistically significant (two-tailed t-test, $p > .05$). Because groups exhibited approximately 50% variability in starting weights, body weight change was analyzed in several ways.

Table 4. Daily Food Allotments" According to Diet, Body Size, and Monkey Species

Body Weight (Kg)	Control (g)	Degree of restriction − 10% (g)	− 20% (g)	− 30% (g)
Rhesus Monkeys				
1.5	130	117	104	91
2.0	137	124	111	98
2.5	137	124	111	98
3.0	150	137	124	104
3.5	156	143	130	111
4.0	176	163	143	124
4.5	189	176	156	130
5.0	195	176	156	137
5.5	215	195	176	150
6.0	221	202	182	156
6.5	234	215	189	163
7.0	241	221	195	169
7.5	247	221	202	176
8.0	254	234	208	176
8.5	260	234	208	176
9.0	260	234	208	182
12.0	280	254	228	195
15.0	299	273	241	215
18.0	319	292	260	228
Squirrel Monkeys				
0.20	13	12	11	10
0.25	16	15	13	12
0.30	23	21	19	16
0.35	26	24	21	18
0.40	31	28	25	22
0.45	36	33	29	25
0.50	41	37	33	29
0.55	44	40	36	31
0.60	46	42	37	33
0.65	47	43	38	34
0.70	48	44	39	34
0.75	48	44	39	34
0.80	48	44	39	34
0.85	50	45	40	35
0.90	51	46	41	36
0.95	54	49	44	38
1.00	57	52	46	40
1.05	60	54	48	42
1.10	63	57	51	44
1.15	66	60	53	46
1.20	69	63	56	49

[a]Some early minor adjustments were made to increase levels for the lightest monkeys and decrease levels for the heaviest monkeys.

First, a 2 (diet) by 13 (month) analysis of variance (ANOVA) with repeated measures on the last factor was conducted separately for juvenile and adult groups of each species. The results of these analyses revealed no significant main effects of diet (ps $> .05$) but significant main effects of months (ps $< .0001$) to substantiate the observed increases in body weight. Most importantly, as hypothesized, significant diet by month interactions were observed in the results

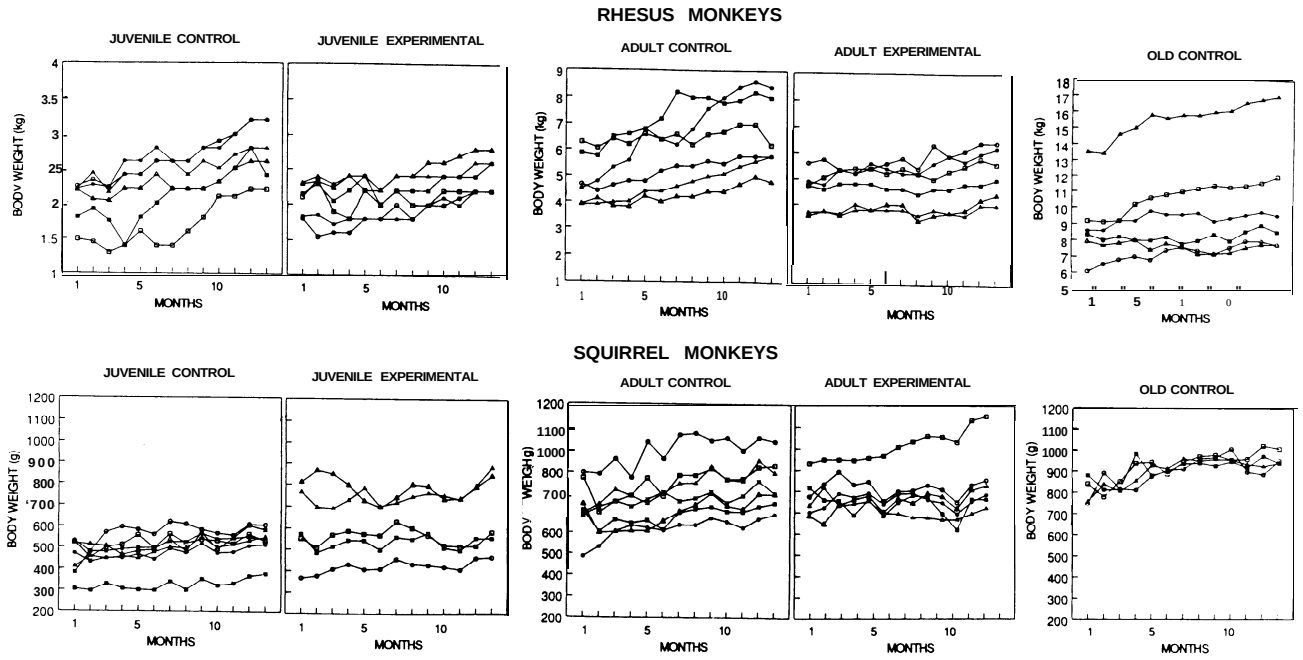

Figure 1. Individual body weights of monkeys.

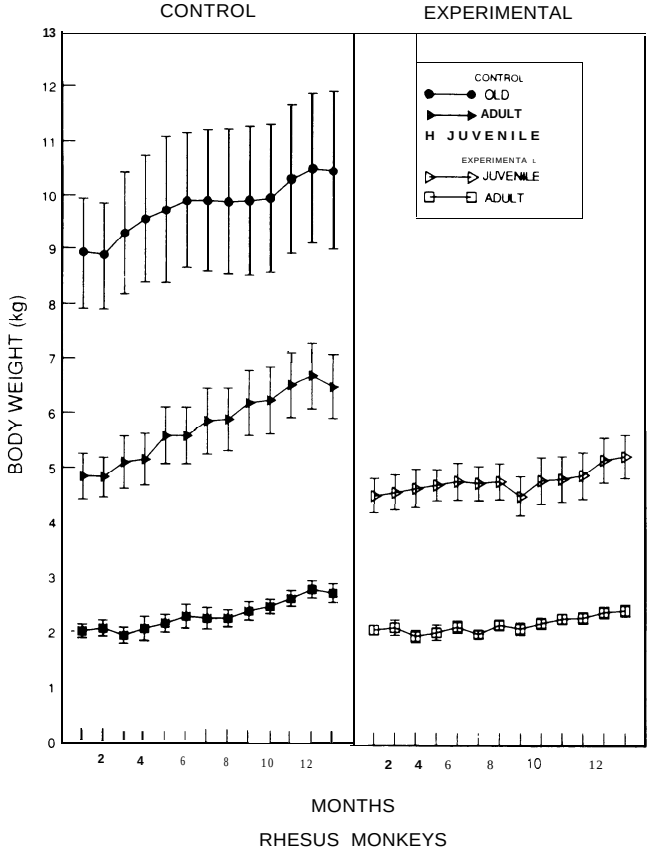

Figure 2. Mean *(SEM)* body weights of rhesus monkeys.

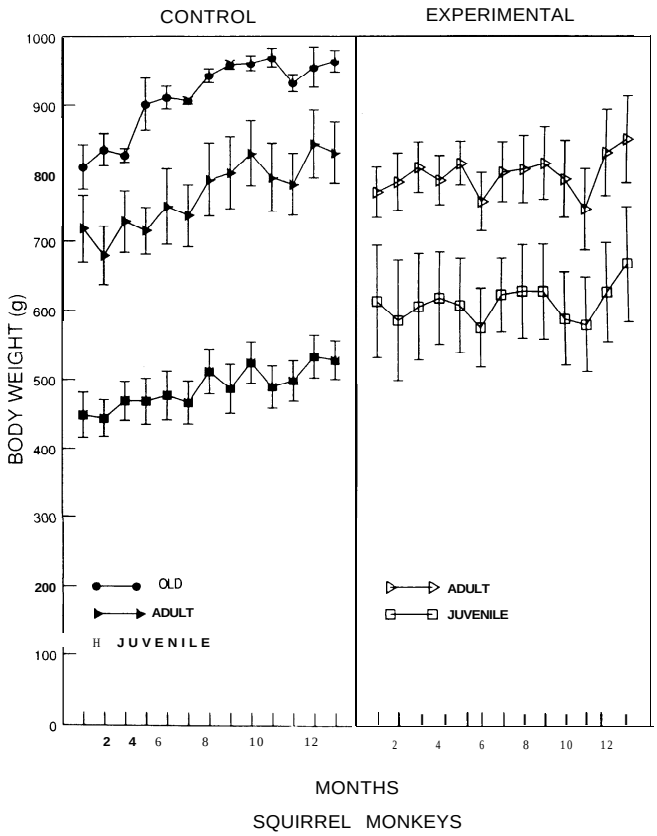

Figure 3. Mean *(SEM)* body weights of squirrel monkeys.

of these analyses to indicate retarded body weight increase among experimental groups (juvenile rhesus: F[12,120]= 4.36, $p<.0001$; adult rhesus: $F[12,120] = 4.97$, $p<.0001$; juvenile squirrel: $F[12,120] = 1.89$, $p<.05$; adult squirrel: $F[12,132] = 3.42$, $p<.0003$).

Longitudinal change in body weight of individuals was

also analyzed for diet and age effects. Linear regression analysis was first applied to compute the individual slopes of body weight as a function of time for each monkey. Reasonable linear fits were made with body weight data from the control groups. Median Pearson product-moment correlation coefficients for individual body weight gain within groups ranged from 0.75 for juvenile squirrel monkeys to 0.87 for juvenile rhesus monkeys. Among the experimental groups, the lowest median coefficient within any group was 0.16 for the juvenile squirrel monkeys, while the highest was 0.76 for juvenile rhesus monkeys.

Representing absolute changes in body weight, the individual slopes were first submitted to a 2 (age: juvenile vs adult) by 2 (diet: control vs experimental) ANOVA within each species. Mean (*SEM*s) weight-based growth rates are presented in Figure 4 (A and B). The old group was omitted from this analysis because their inclusion would not permit a factorial analysis. Adults displayed a higher rate of body weight increase compared to juveniles, and monkeys on the restricted diet were gaining weight more slowly compared to controls. Among rhesus monkeys, the results of the ANOVA confirmed the significant main effects of age, $F(1,20) = 5.24$, $p < .05$, and diet, $F(1,20) = 9.94$, $p < .01$, with no significant interaction, $F(1,20) = 2.75$, $p > .05$. Among squirrel monkeys, statistical confirmation was made with respect to the main effect of diet, $F(1,20) = 8.03$, $p < .02$; but neither the main effect of age nor the interaction was significant, $F(1,20) = 1.01$, $p > .05$, and $F(1,20) < 1.0$, respectively.

When the absolute body weight gain of the experimental groups was expressed as a percentage of that of controls, the degree of restriction could be assessed. Among rhesus monkeys. the body weight increase of the restricted groups was 48% and 29% of that for the juvenile and adult control groups, respectively. For the squirrel monkeys, the body weight increase of the experimental juvenile and adult groups was 35% and 24% of their respective control group rates. Diet restriction appeared to have a greater impact on body weight gain among squirrel monkeys compared to rhesus counterparts.

To compute the relative body weight gain for each monkey, individual slopes of body weight gain were divided by baseline weight, which was the mean of the first two monthly weighings prior to dietary restriction. These estimates were then submitted to a 2 (age: juvenile vs adult) by 2 (diet: control vs experimental) ANOVA for each species. Again, the old groups were omitted from this analysis.

Mean (*SEM*s) relative growth rates in body weight were generally higher among rhesus compared to squirrel monkeys, and the effect of diet was marked (Figure 4, C and D). In both species, the ANOVAs yielded significant main effects of diet, $F(1,20) = 9.20$, $p < .001$, for rhesus monkeys; $F(1,20) = 19.55$, $p = .0003$ for squirrel monkeys. Neither the main effect of age nor the age by diet interaction proved significant in either species ($ps > .05$).

When the relative rates of body weight gain in experimental groups were expressed as a percent of that for appropriate controls, a consistency in diet effect emerged across age groups in both species. The degree of relative body weight gain among restricted rhesus monkeys was 46% and 49%

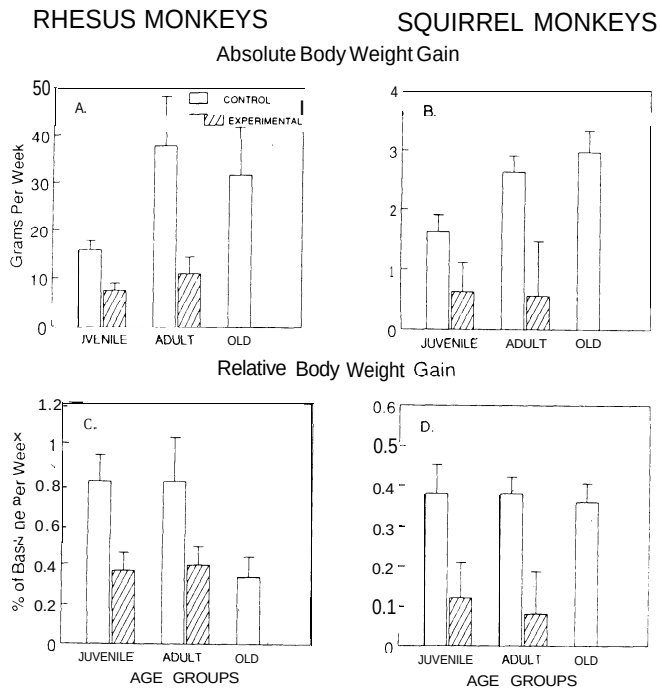

Figure 4. Mean *(SEM)* absolute body weight gain and relative body weight gain of rhesus (A, C) and squirrel monkeys (B, D).

that of juvenile and adult controls, respectively. Among squirrel monkeys, these estimates were 32% and 20% for juvenile and adult groups, respectively. Thus, when assessed on a relative basis, the degree of reduction in body weight gain appeared greater among squirrel monkeys. However, within both species no significant age difference in the effect of the diet regimen on relative body weight gain was apparent.

Changes in body weight in the old group were compared to those of the juvenile and adult control groups in one-way ANOVAs separate for species in both absolute and relative body weight measures. Results of the ANOVAs on absolute body weight increase revealed a significant age-related increase in squirrel monkeys, $F(2,14) = 5.64$, $p < .01$ (Figure 4B); however, the effect of age was not significant for rhesus monkeys, $F(2,15) = 1.81$, $p > .05$ (Figure 4A). The results of ANOVAs on relative body weight gain indicated no significant effect of age in either rhesus, $F(2,15) = 2.91$, $p > .05$, or in squirrel monkeys, $F(2,14) < 1.0$. Thus, relative body weight gain appeared equivalent across age groups in both species. However, it should be noted that the relative increase in body weight of old rhesus monkeys was half that of the younger groups. It is also important to note that old monkeys of both species were gaining body weight while consuming these diets.

Food Intake

Table 5 presents food intake data obtained over a one-week sampling period at the approximate midpoint of the first year of the study. Absolute food consumption was reduced by 23 and 24% for juvenile and adult experimental rhesus monkeys, respectively. The corresponding reduc-

tions for the same groups of squirrel monkeys were 22 and 24%. Although diet-restricted rhesus monkeys consumed their entire food allotment, squirrel monkeys in the experimental group did not consume 7-12% of their food. Because food was left unconsumed by control animals of both species, it appears that these monkeys were ad libitum fed. The largest amount of uneaten food was recorded for old monkeys of both species.

Hematology and Blood Chemistry

The results of the hematology and blood chemistry assays conducted for seven occasions over a IO-mo interval are summarized in Tables 6-7 and Figures 5-10. All assayed constituents are listed in the tables; however, several constituents (banded neutrophils, monocytes, atypical lymphocytes, and direct and indirect bilirubin) were not analyzed statistically because their values rarely exceeded zero. To permit any desired comparison to standard values from the same or different species, the mean ($\pm SD$) values for all other constituents are provided for each species.

Diet effects on hematological and blood chemical variables were assessed by conducting two-tailed t-test comparisons ($p < .05$) between control and experimental animals within each age group. Only data for those comparisons that were significant on at least one of the last two measurement occasions are listed in the tables. This logic was applied to identify those variables which indicated the potential for lasting changes that appeared relatively early in the study. The age effects listed represent those variables that exhibited significant differences (two-tailed t-test comparisons, $p < .05$) between any two age groups on at least two occasions, and that two or more age differences were consistent, i.e., there are no reversals in the direction of the significant age

Table 5. Effect of Age and Diet on Food Consumption

Age	Diet	N	Food Consumed (% of Total Allotment)	Food Consumed (% of Absolute Control Group Consumption)
		Rhesus Monkeys		
Juvenile	Control	6	91 ± 5	100 ± 6[a]
	Experimental	6	100 ± 0	77 ± 0[a]
Adult	Control	6	91 ± 4	100 ± 5[b]
	Experimental	6	100 ± 0	76 ± 0[b]
Old	Control	6	79 ± 9	100 ± 11
		Squirrel Monkeys		
Juvenile	Control	7	83 ± 2	100 ± 2[c]
	Experimental	5	93 ± 2	78 ± 2[c]
Adult	Control	7	82 ± 4	100 ± 5[d]
	Experimental	6	88 ± 3	76 ± 2[d]
Old	Control	4	68 ± 6	100 ± 9

Notes. Values are the mean *(SEM)* for the indicated numbers of animals. [a,b,c,d]significantly different from corresponding letter group ($p < .01$ by unpaired t-test).

Table 6. Summary of Hematology Results

Abbreviation	Parameter	Units	Species[a]	Mean	S D	Diet Effects[b]	Age Effects
RBC	Red blood cells	10^6/mm^3	RH	5.3	0.5		O > A = J
			SQ	7.1	0.7		
HGB	Hemoglobin	g/dl	RH	12.7	1.2		O > A = J
			SQ	13.7	1.5		
HCT	Hematocrit	%	RH	38.5	3.9		O > A = J
			SQ	42.4	4.3		
MCV	Mean corpuscular volume	μ^3	RH	73.8	3.8		
			SQ	59.2	3.9		
MCH	Mean corpuscular hemoglobin	pg	RH	24.4	1.7		
			SQ	19.5	1.5		
MCHC	Mean corpuscular hemoglobin conc	%	RH	32.9	1.3	JE = 34.5 > JC = 33.0 (11)	
			SQ	32.8	1.3		
WBC	White blood cells	10^3/mm^3	RH	6.8	2.7		O > A = J
			SQ	6.7	2.3		J = O > A
POLYs	Polymorphonuclear leukocytes	%	RH	41.2	15.1	JE = 36.7 > JC = 23.7 (8) JE = 47.5 > JC = 34.4 (11) AC = 42.3 > AE = 30.0 (8)	
			SQ	42.1	15.3	JC = 38.6 > JE = 18.6 (8)	
LYMPH	Lymphocytes	%	RH	51.8	15.4	JC = 72.0 > JE = 58.3 (8) JC = 56.6 > JE = 44.2 (11) AE = 63.7 > AC = 51.3 (8)	J = A > O
			SQ	51.5	15.5		J > A > O
MONOS	Monocytes	%	RH	2.2	1.9		
			SQ	2.3	2.0		
EOSIN	Eosinophils	%	RH	3.3	4.1		O > A = J
			SQ	3.2	3.3		O > A > J

[a]RH = Rhesus; SQ = Squirrel; [b]J = Juvenile; A = Adult; 0 = Old; C = Control; E = Experimental; (#) = Month Observed.

Table 7. Summary of Blood Chemistry Results

Abbreviation	Parameter	Units	Species[a]	Mean	*SD*	Mean Diet Effects[b]	Age Effects
LDH	Lactic dehydrogenase	IU/L	RH	586.0	15.5	AC = 415 > AE = 329 (11)	
			SQ	283.0	141 .o		
SGOT	Serum glutamic oxalacetic transaminase	IU/L	RH	64.3	33.3		J > A = O
			SQ	187.0	88.0	JC = 221 > JE = 144 (11)	J > A = O
SGPT	Serum glutamic pyruvic transaminase	IU/L	RH	42.6	28.6		J > A = O
			SQ	173.0	125.0		J > A > O
ALK PHOS	Alkaline phosphatase	IU/L	RH	452.0	232.0		J > A > O
			SQ	384.0	286.0		J > A = O
T-BILI	Total bilirubin	mg/dl	RH	0.13	0.05		
			SQ	0.20	0.21		
T-PRO	Total protein	g/dl	RH	7.1	0.6		A > O > J
			SQ	6.9	0.9		O > A > J
ALBU	Albumin	g/dl	RH	4.3	0.5	AE = 5.1 > AC = 4.6 (11)	A > J > O
			SQ	3.4	0.5		
GLOB	Globulin	g/dl	RH	2.9	0.7		O > A > J
			SQ	3.6	1.0		O > A > J
A/G RATIO	ALBU/GLOB Ratio		RH	1.6	0.4		J > A > O
			SQ	1.0	0.2		J > A > O
CHOL	Cholesterol	mg/dl	RH	138.0	29.1		J > A > O
			SQ	152.0	31.1		
GLU	Glucose	mg/dl	RH	66.3	22.2		
			SQ	66.2	21.3		
BUN	Blood urea nitrogen	mg/dl	RH	18.9	6.4		J > A = O
			SQ	23.2	7.1		J = A > O
CREAT	Creatinine	mg/dl	RH	0.95	0.18		O > A > J
			SQ	0.67	0.15	JE = 0.76 > JC = 0.59 (8)	O > A > J
BUN/CREAT	BUN/CREAT Ratio		RH	20.7	8.4		J > A = O
			SQ	36.3	12.9		J = A > O
URIC	Uric acid	mg/dl	RH	0.12	0.12		
			SQ	0.19	0.33		
CALC	Calcium	mg/dl	RH	9.3	0.5	JC = 9.4 > JE = 8.9 (1 1)	J = A > O
			SQ	8.5	0.5		
PHOS	Phosphates	mg/dl	RH	5.2	1.3	JC = 7.5 > JE = 5.8 (11)	J > A > O
			SQ	4.1	1.5		J > A > O

[a]RH = Rhesus; SQ = Squirrel; [b]J = Juvenile; A = Adult; 0 = Old; C = Control; E = Experimental; (#) = Month Observed.

difference across measurement occasion. Diet groups have been combined for these age comparisons because there was rare occasion of diet effects occurring during more than one month. Both diet and age comparisons were made only if there were at least four observations in each group for that occasion. Variables for which significant age effects were noted are also presented in Figures 5– 10.

This statistical strategy of applying multiple t-tests was conducted with awareness of the risks involving greatly increased Type I error rate, that is, concluding significance that arises by chance alone. This risk was accepted because (a) the fact of many missing data points across the measurement occasions for all variables made it impossible to conduct a repeated measures ANOVA; (b) since data on hematology and blood chemistry were being used to identify possible detrimental effects of the nutritional manipulation, we wanted to apply the most liberal analysis initially; and (c) the lack of statistical significance emerging from this liberal analysis might permit the exclusion of selected variables from future collection or assessment. All significant findings reported here will be repeated in future analyses to assure their reliability.

Diet Effects

Hematology. — From a possible 392 total comparisons, only 20 significant diet effects were observed (Table 6). With only two exceptions, these effects emerged on only one measurement occasion with 14 out of 20 observed on one or both of the last two measurement occasions as shown in Tables 6 and 7. On only rare instances were the diet effects consistent across age groups or species.

Regarding erythrocyte parameters, the only comparison of interest was observed for MCHC, which was higher among juvenile rhesus experimental monkeys compared to controls on the last measurement occasion after 7 months on

30% dietary restriction. No erythrocyte parameter was significantly affected in the squirrel monkeys, but many comparisons could not be made because of insufficient sample sizes.

Regarding leukocyte parameters, several significant, but again inconsistent, diet effects were noted. POLYS were observed to be higher among rhesus adult control monkeys compared to experimental control monkeys on the sixth measurement occasion (month 8). This was opposite the pattern among juvenile rhesus monkeys, where experimental animals had higher POLYS counts compared to control animals on the same occasion, and this difference was maintained in the juvenile rhesus experimental group on the last measurement. The pattern in juvenile squirrel monkeys was similar to that observed among adult rhesus. On the next to last measurement (month 8), the percentage of POLYS was higher in control animals compared to experimental animals in this age group. Juvenile rhesus experimental monkeys had lower percentages of LYMPH compared to that control group on the last two occasions; however, this pattern contrasted to the higher level observed among adult rhesus experimental animals compared to their control animals on occasion 8. In summary, little consistency existed across age groups and species with respect to diet effects on the hematological parameters examined. However, the values obtained all appeared to be within normal ranges for the species (Abee, 1985; McClure, 1975).

Blood *chemistry.*— Several diet effects on blood chemistry variables emerged on the last two measurements which render them important candidates for future analysis (Table 7). LDH activity for adult rhesus monkeys was higher in the control group compared to the experimental group. In the same age group of rhesus monkeys, ALBU concentration was higher in experimental compared to control animals. Among juvenile rhesus groups, CALC and PHOS concentrations were higher among control compared to experimental groups. Diet effects among squirrel monkeys were observed for only two variables. Juvenile control groups exhibited higher activity of SGOT compared to experimental groups. Juvenile experimental animals exhibited higher CREAT concentrations compared to control animals on the next to last occasion (month 8).

Age Effects

As stated above, control and experimental animals from the same ages were grouped to assess age effects because the diet effects were infrequent.

Hematology.— Among rhesus monkeys very consistent age effects on RBC, HGB, and HCT were observed (Figure 5). Old monkeys had higher values for these parameters compared to juvenile and adult monkeys, which did not differ significantly. This observation was consistent across all measurement occasions except for month 8, when RBC and HGB for the adult rhesus group were elevated to the value of the old group, but then returned to their previous levels on the last occasion. The age patterns among squirrel monkeys for RBC, HGB, and HCT were much less consistent, and generally nonsignificant. During the last few measurements, though, all the parameters appeared to be lower in juveniles compared to adults of this species.

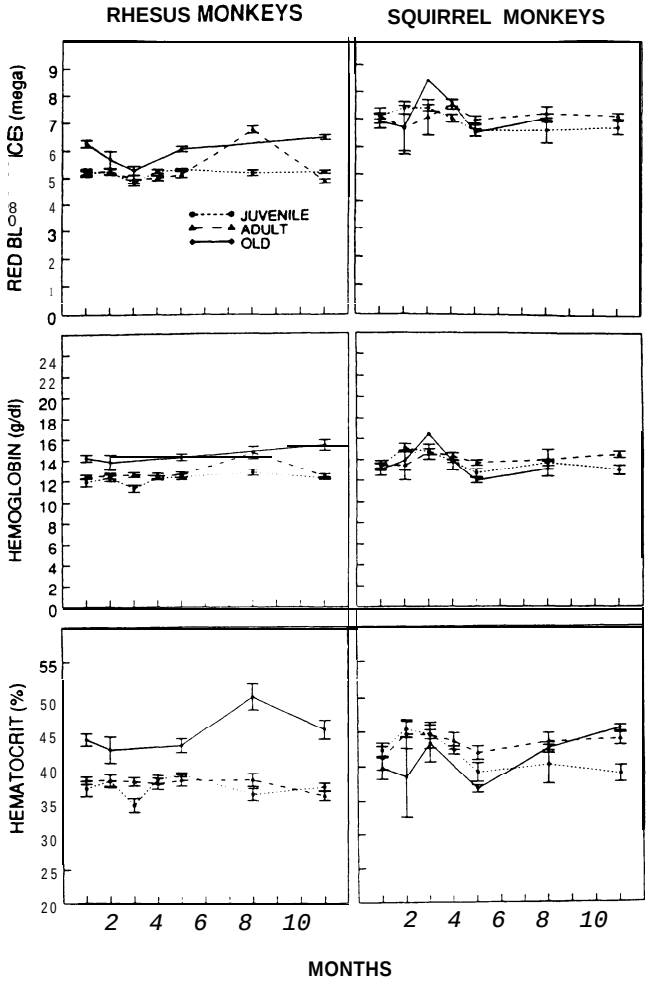

Figure 5. Mean *(SEM)* erythrocyte measures (red blood cells, hemoglobin, hematocrit) in rhesus and squirrel monkeys.

Regarding leukocyte parameters shown in Figure 6, old rhesus monkeys had higher values of WBC and EOSIN compared to juvenile and adult groups, which were similar to the pattern of erythrocyte results. This difference was generally consistent across all measurement occasions. The age difference in EOSIN was also recorded for squirrel monkeys, and this parameter also appeared to increase between juvenile and adult groups. This latter trend was also evident among rhesus monkeys. However, in contrast to the pattern observed in WBC among rhesus monkeys, it appeared that on at least two early measurements, WBC levels among juvenile squirrel monkeys were higher compared to adult and old groups. Regarding LYMPH concentrations, age differences were generally consistent across species. Among rhesus monkeys, LYMPH concentrations were clearly lower among old groups compared to juveniles and adults. A similar age pattern in LYMPH concentration, however, was not observed among squirrel monkeys until the last few measurements.

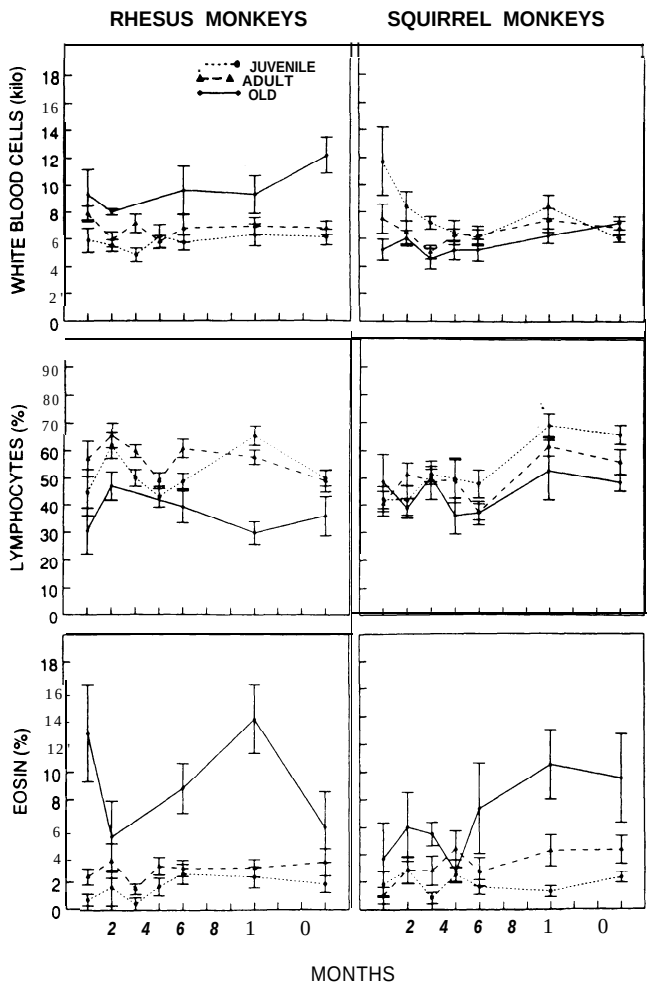

Figure 6. Mean *(SEM)* leukocyte measures (white blood cells, lymphocytes, eosinophils) in rhesus and squirrel monkeys.

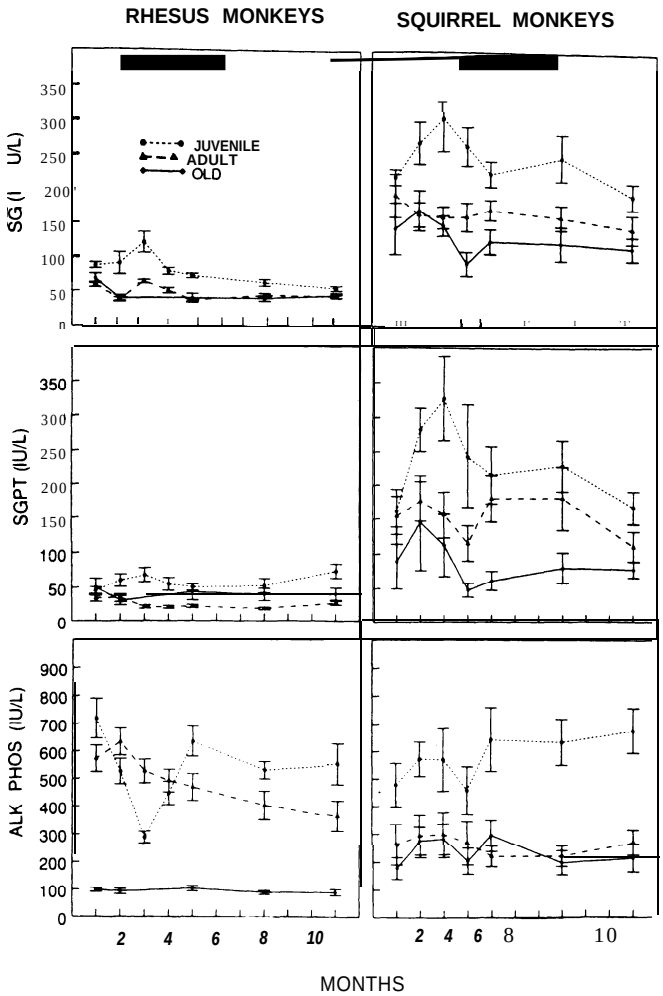

Figure 7. Mean *(SEM)* serum enzyme levels (serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase) in rhesus and squirrel monkeys.

Blood chemistry. — Age effects observed for SGOT, SGPT, and ALK PHOS were very consistent (Figure 7). In general for all three enzymes, concentrations declined as a function of age in both species. For SGOT the age effect appeared more as a developmental change between juvenile and older groups. The same developmental trend was true for SGPT among rhesus monkeys, although the values for the old group approached those for juvenile animals on a couple of occasions. Values for the adult group were consistently lower compared to the juvenile group. The pattern in SGPT concentrations appeared more uniform among squirrel monkeys with the appearance of a consistent age-related decline in this enzyme. For ALK PHOS the age decline was generally uniform and large for rhesus monkeys. In contrast, the age effect for ALK PHOS among squirrel monkeys appeared to be developmental.

T-PRO concentrations showed an inconsistent age pattern among rhesus monkeys (Figure 8). Values were the lowest for juvenile monkeys. However, adults of this species exhibited the highest values for several months with the old group at an intermediate level. For squirrel monkeys T-PRO concentrations increased consistently with age.

ALBU and GLOB concentrations as well as their ratio exhibited significant age effects primarily in rhesus monkeys (Figure 8). For GLOB, marked and consistent age-related increases in concentrations were observed in both species. For ALBU, no significant age pattern was observed for squirrel monkeys. For rhesus monkeys, the oldest group generally had the lowest concentrations of ALBU; however, the highest levels were observed among adults while the juvenile group exhibited intermediate values but close to that of adults. Age differences in the A/G ratio were observed in both species; however, the age-related decrease in A/G ratio was more consistent over time among rhesus monkeys.

Parameters of kidney function also exhibited significant age effects that were generally evident across species (Figure 9). CREAT concentrations showed the most consistent pattern of an age-related increase in both species. In contrast, the age pattern for BUN was more complex. Among rhesus monkeys, the highest BUN values were reported for juvenile groups with lowest for adults. Among squirrel monkeys, again juveniles had the lowest values but generally equivalent to the levels of adult monkeys, and old monkeys exhibited the lowest values on several occasions. For the BUN-

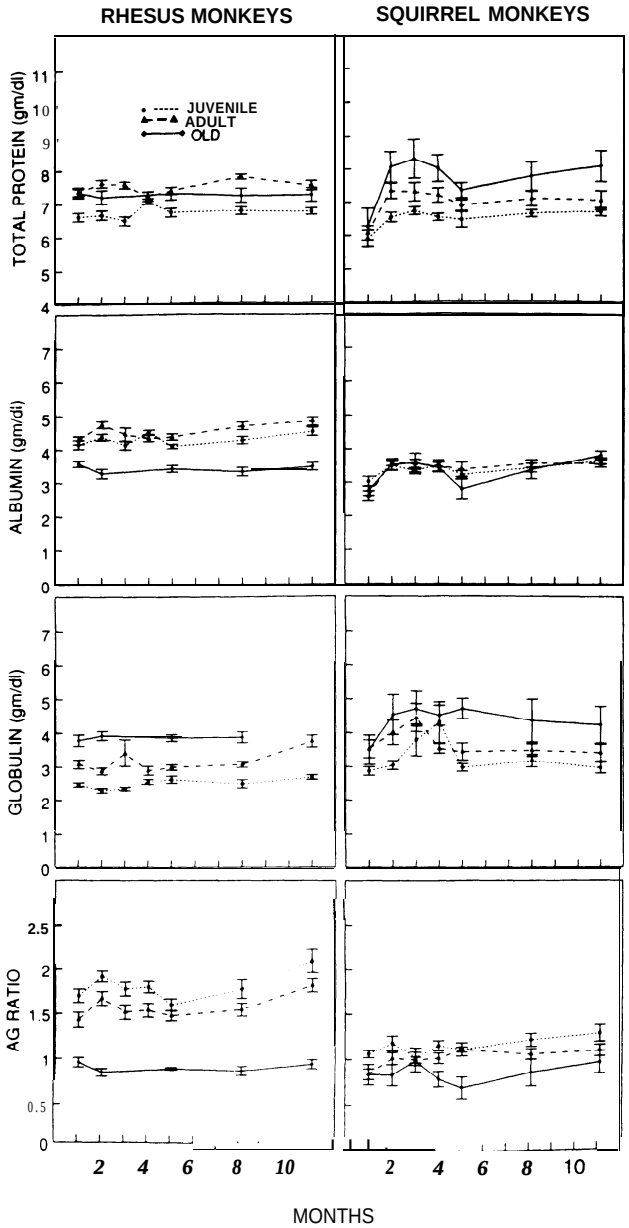

Figure 8. Mean ***(SEM)*** serum protein levels (total protein, albumin, globulin, albumin/globulin ratio) in rhesus and squirrel monkeys.

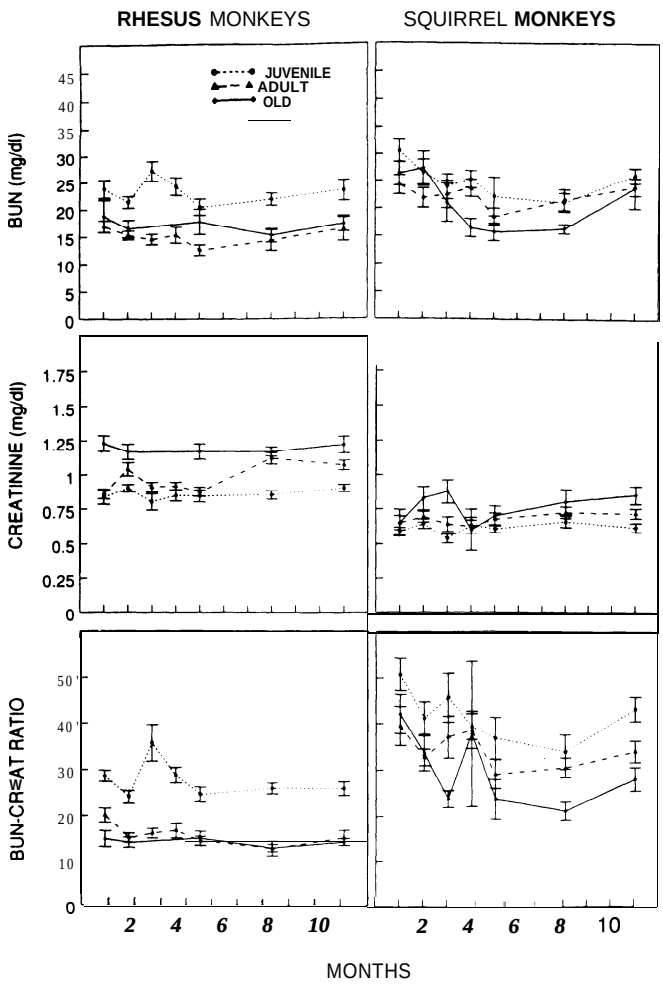

Figure 9. Mean ***(SEM)*** serum levels of blood urea nitrogen, creatinine, and their ratio in rhesus and squirrel monkeys.

CREAT ratio, a developmental trend was observed among rhesus monkeys. Juvenile monkeys in this species were consistently higher in this ratio compared to adult and old groups. A uniform age-related decrease in this ratio was somewhat more evident among squirrel monkeys.

Analysis of serum concentrations for CHOL and the minerals CALC and PHOS also revealed significant age differences (Figure 10). Over the last few measurement occasions, an age-related decrease in serum CHOL emerged among rhesus monkeys. CALC concentrations in this species also exhibited an age-related decrease between adult and old groups. For PHOS concentrations, a generally consistent age-related decrease was observed in both species.

DISCUSSION

The major objective of this study was to assess the feasibility of implementing dietary restriction in nonhuman primates to determine its relevance as an experimental manipulation of aging processes. This challenge was difficult in view of the general lack of information on appropriate nutrition for captive primate species (National Research Council, 1978). A previous study had indicated the feasibility of studying the effects of nutritional manipulation on aging in *Macaca nemestrina* (Short et al., 1987); however, this investigation altered primarily fat content without deliberately reducing caloric content. Other primate nutrition studies have focused on manipulation of specific diet components, e.g., fiber (Morin et al., 1978).

The critical question confronted was whether a nutritionally adequate diet suitable to each species could be provided to permit feeding to experimental animals at a level 30% less than recommended control values without detriment to general health. As indicated in rodent studies, the goal was to achieve dietary restriction without malnutrition (Weindruch and Walford, 1988). This goal required careful monitoring

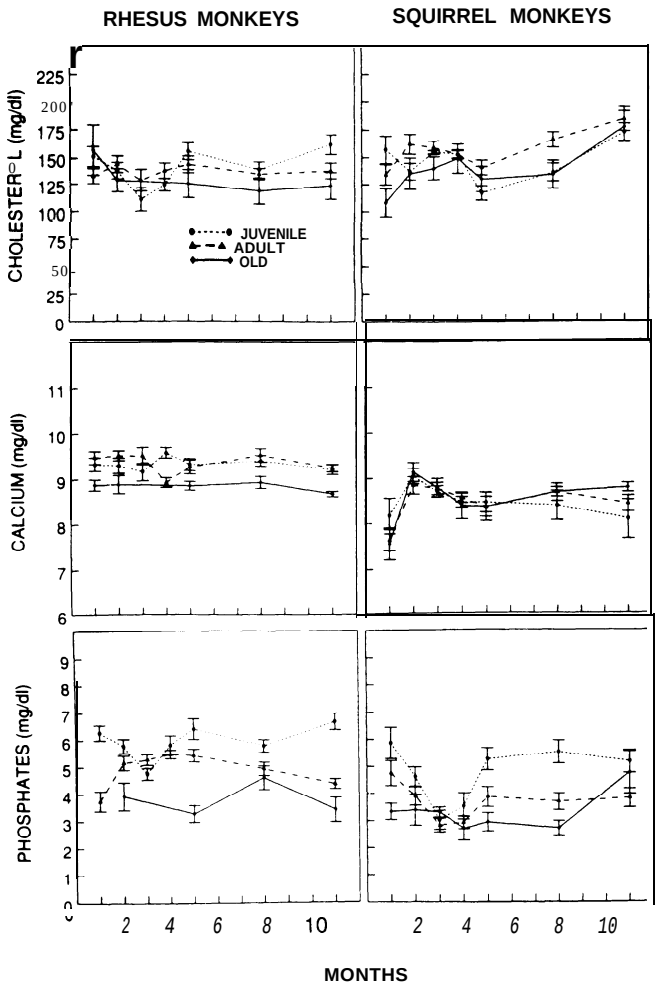

Figure 10. Mean *(SEM)* serum levels of cholesterol, calcium, and phosphates in rhesus and squirrel monkeys.

of the monkeys, especially during the early transitional phases of the experiment. Adjustments were necessary early in the study, particularly with respect to the amount of food provided juvenile monkeys of both species. However, based on analysis of body weight change in addition to the normality of the hematology and blood chemistry data obtained from the first year of this study, we conclude that the current results support the feasibility of further analysis of this dietary restriction regimen in the present species.

Body Weight and Food Intake

Unquestionably the dietary restriction regimen has been successful in altering the rate of body weight gain of juvenile and adult monkeys in both species. It should be noted that food intake studies reveal an actual reduction of 22–24% rather than the 30% ideally sought on the basis of allotment. Part of the discrepancy may be due to mechanical factors such as food loss through the cage or behavioral peculiarities such as playing with food and untidy eating habits. Further dietary reductions might be considered in light of the level of restriction proven successful (up to 50%) in rodent studies (Weindruch and Walford, 1988). Despite these variables, however, a substantial reduction in intake has been clearly reflected in reduced body weight gain.

Equally important is the observation that the diet for each species appears adequate for normal growth in young monkeys. All control groups are gaining body weight. This conclusion can be further bolstered by projecting the absolute changes in body weight for the juvenile control groups over 3 years for both species. Assuming a continued linear absolute increase in body weight at this stage of development (Boume, 1975; Portman,1970), the projected increase for rhesus monkeys over 3 years would be about 2.5 kg, and for squirrel monkeys, about 0.25 kg, which would place them at the mean level of their respective adult groups at the beginning of the study. One present concern, however, is the increase in body weight of the old groups. If this trend were to continue, it might signal that the diet was overnutritious relative to the exercise level. Many adult rhesus monkeys in laboratory environments can develop frank obesity and diabetes at older ages (Hanson and Jen, 1979).

In the juvenile and adult groups of both species, dietary restriction has slowed body weight gain. Among rhesus monkeys, the divergence between groups is very clear when examining mean body weights. This divergence is less evident when examining the mean body weights of squirrel monkey groups. However, when examining the slopes of individual body weight gain across the first year of this study, the divergence between control and experimental groups is obvious. Some disproportionality of the diet effect appears across age groups when absolute body weight gain is considered, but then the view of relative body weight gain indicates an equivalent effect across age groups. On this relative basis, it appears that the diet effect is greater among squirrel monkeys compared to the effect among the rhesus. Linear projections of the current absolute rates of body weight gain for juvenile experimental animals in both species over 3 years would yield estimates indicating relatively small monkeys for their ages. Among rhesus juveniles, these monkeys should have a mean body weight of about 3.7 kg compared to 5.2 kg for controls. Over 3 years, juvenile squirrel monkeys on dietary restriction would be expected to gain somewhat less than 100 g. Given the range of body weights within juvenile diet group, a significant difference between control and experimental groups of this species would likely still not exist even after 3 years.

Hematology and Blood Chemistry

From the analysis of the hematology and blood chemistry data, we found no evidence of a detrimental effect of dietary restriction. With the exception of the juvenile groups that required some early adjustments to their allotted intakes, all monkeys now appear to be healthy. The values obtained in this clinical screen were within normal ranges for both rhesus (McClure, 1975) and squirrel monkeys (Abee, 1985). For rhesus monkeys many of these values could be assessed against those of an earlier study that compared adult (6-14 yr) to aged (15-28 yr) groups (Kessler et al., 1983).

Diet effects on the extensive list of hematology and blood chemistry parameters examined were infrequent and inconsistent across age and species. The lack of diet effects might be due to small sample sizes and interindividual variability

or the moderate degree of diet restriction imposed. Perhaps a longitudinal analysis will be more sensitive to group differences. Addition of new data samples collected quarterly will permit such an analysis. Alternatively, the amount of time thus far devoted to dietary restriction or the severity of diet restriction may be insufficient to produce group differences in these parameters for these long-lived species. Short-term dietary restriction (5-6 weeks) in rats has been shown to affect several parameters in our battery, including erythrocyte parameters (Pickering and Pickering, 1984) and glucose, uric acid, and fats (Sachan and Das, 1982). Diet restriction is also known to decrease several leukocyte parameters in mice (Weindruch and Walford, 1988). In the present study lymphocyte concentrations were reduced among experimental juvenile rhesus monkeys compared to controls but not among squirrel monkeys; however, lymphocyte concentrations were increased among adult experimental rhesus monkeys compared to control values.

In addition to health monitoring, application of the hematology and blood chemistry battery also permitted assessment of potential markers of development and aging that might be used to determine whether dietary restriction altered the rate of development and aging when analyzed over longer time periods (Short et al., 1987). Many candidate markers emerged in the first year of this study with some parameters appearing more stable than others.

The highly related erythrocyte parameters — RBC, HGB, HCT — all exhibited an age-related increase between adult and old rhesus monkeys. This observation paralleled that previously reported for male pigtailed macaques (Short et al., 1987) and was similar to values obtained for aged rhesus monkeys (Kessler et al., 1983).

WBC also increased with age among rhesus monkeys, which had not been reported in the earlier study of pig-tailed macaques (Short et al., 1987) or in humans (Adler and Nagel, 198 1). This parameter appears inconsistent across species since among squirrel monkeys the juveniles had the highest concentrations of WBC. Among the other leukocyte parameters, LYMPHS and EOSIN exhibited consistent age effects in both species. Eiosinophilia increased with age, which had been observed in a previous study of male and female rhesus monkeys (Kessler et al., 1983). This observation might result from longer exposure to parasitic infections rather than to aging per se. However, the age-related decrease in LYMPH concentration would appear less sensitive to the exposure issue.

From the blood chemistry analysis, SGOT, SGPT, and ALK PHOS concentrations appear to be excellent candidates for examining effects of dietary restriction on either development or aging. These three enzymes appear to reflect developmental differences between juvenile and adult groups in both species. ALK PHOS in rhesus monkeys and SGPT in squirrel monkeys reflect the best potential markers of aging from this group of variables. The previous study of pig-tailed macaques did not report any age differences in ALK PHOS levels (Short et al., 1987). No significant age-related decline in ALK PHOS was reported between adult and aged male rhesus monkeys although a decline was observed in females (Kessler et al., 1983). The high levels of ALK PHOS among juvenile animals may reflect the normal bone growth activity (osteoblasts secrete ALK PHOS into the osteoid matrix), which is diminished as a function of age.

High levels of SGOT and SGPT are usually indicative of liver damage in clinical screens. No significant difference between adult and aged rhesus monkeys was observed for either parameter in an earlier study (Kessler et al., 1983). The high levels observed in juvenile animals in the present study are probably due to developmental factors. In this case, elevated SGOT could reflect greater levels of locomotor activity resulting in increased production in muscle (Benjamin, 1978). SGPT appears to be more specific to liver function with the higher juvenile levels possibly representing a greater degree of fatty metamorphosis (Benjamin, 1978).

Regarding blood proteins, T-PRO may be a candidate for assessing development in both species and aging in squirrel monkeys. Short et al. (1987) reported no significant age effects on this parameter in pig-tailed macaques, but Gillibrand et al. (1980) noted a slight age-related decrease in human males for total protein. This measure may reflect the age-related increase in GLOB production observed in both species which parallels findings for age effects on GLOB in human males (Borkan and Norris, 1980; Gillibrand et al., 1980), rhesus monkeys (Kessler et al., 1983), and miniature swine (Tumbleson et al., 1976). ALBU concentration appears as a stable marker of aging among rhesus monkeys. A monotonic decline with age was also observed in pig-tailed macaques (Short et al., 1987), human males (Borkan and Norris, 1980; Gillibrand et al., 1980), and in miniature swine after 11 mo of age (Tumbleson et al., 1976). The different direction of the age effects in ALBU and GLOB is reflected in the great stability in age effects on the A/G ratio among rhesus monkeys and to some extent among squirrel monkeys.

Kidney function tests also offered some possible markers of development and aging to examine in future studies. In rhesus monkeys the developmental decline in BUN between juvenile and adults is a good candidate to follow. No age effects were noted in rhesus (Kessler et al., 1983) or pig-tailed macaques (Short et al., 1987), but the developmental decline was reported for miniature swine followed by an age-related increase after 8 mo of age (Hutcheson et al., 1979). In human males an increase in BUN after 60 yrs of age has been noted (Gillibrand et al., 1980), and a positive correlation with age in females has also been reported (Webster and Logie, 1976). The major age difference in BUN for squirrel monkeys in the present study appeared between adult and old groups. Developmental and aging patterns were clearly evident in CREAT concentrations in both species. No age effects were reported for this parameter among rhesus (Kessler et al., 1983) or pig-tailed macaques (Short et al., 1987). An age-related increase in CREAT has been reported for human males (Gillibrand et al., 1980). When the ratio of BUN to CREAT was considered in the present study, the developmental decline was very evident among rhesus monkeys. Among squirrel monkeys a possible age-related decline was noted.

Regarding blood minerals, both CALC and PHOS emerged as possible markers of aging in rhesus monkeys. The age effect for CALC appeared between adult and old groups.

An age-related decline in serum CALC was reported previously for pig-tailed macaques (Short et al., 1987), human males (Gillibrand et al., 1980), dairy cattle (Tumbleson et al., 1973), and miniature swine (Hutcheson et al., 1979), but not in rhesus monkeys (Kessler et al., 1983). No age effect for CALC emerged in squirrel monkeys in the current study. In contrast, PHOS concentrations exhibited fairly reliable age-related decline in both species although to a less reliable extent among squirrel monkeys. In human males, the age-related decline in phosphorous appears up to 20 years of age and remains stable thereafter (Gillibrand et al., 1980). Both CALC and PHOS may provide measures of bone growth and development to monitor for the effects of diet and aging in the primate species being studied.

Whereas the previous study of pig-tailed macaques (Short et al.. 1987) reported no consistent age effects on CHOL (specific types), a possible age-related decline was noted among rhesus monkeys in the current study, but the effect was not consistent. No such effect was observed in squirrel monkeys. Thus, more observations will be needed to establish the reliability of CHOL as a useful marker of development and aging.

In summary, from a nutritional perspective our initial results indicate that dietary restriction at the current level in juvenile and adult rhesus and squirrel monkeys is feasible. Food intake is sufficiently under control so as to produce differential rates of body weight gain between control and experimental groups at both ages in both species. Additional morphometric measurements are being made to assess further the impact of the diet regimen on development and growth. The analyses of hematology and blood chemistry data suggest no detrimental effects of dietary restriction. Instead, the application of this clinical battery yielded many variables that can be followed as markers of development and aging to assess the effect of the diet manipulation thereon. We recognize that the cross-sectional nature of the current analysis must be verified by longitudinal analysis to rule out possible cohort and secular effects and the influence of genetic heterogeneity. This analysis will be completed as additional data points are added. However, the results of the project, to date, have encouraged the inclusion of other measures to assess the impact of dietary restriction on development and aging and to begin testing in nonhuman primate species possible hypotheses related to biological mechanisms underlying nutritional effects on aging processes.

Acknowledgments

The authors acknowledge the valuable contributions of Ann Higgins from Maryland Medical Laboratories, Baltimore, MD, for management of the hematology and blood chemistry assays; of Rita Wolferman from the Gerontology Research Center for secretarial assistance; of Sharon Davison from Towson State University for data entry and analysis; of Charlene M. Moore from the University of Texas Health Science Center for cytogenetic analysis; and of our scientific advisory committee for the project consisting of Douglas Bowden of the University of Washington, David Harrison of the Jackson Laboratory, Edward Masoro of the University of Texas Health Science Center, and Christian Abee of the University of South Alabama School of Medicine for their helpful discussion and assistance.

The Veterinary Resources Branch of the Division of Research Services, NIH, is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

Address correspondence to Dr. George S. Roth, Gerontology Research Center, Francis Scott Key Medical Center, 4940 Eastern Avenue, Baltimore, MD 2 1224.

References

Abee. C. R. Medical care and management of the squirrel monkey. In: Rosenblum. L. A.: Coe. C.. eds. Handbook of squirrel monkey research. New York: Plenum Press, 1985:447–488.

Adler, W. H .; Nagel, J. E. Studies of immune function in a human population. In: Segre, D.; Smith, L., eds. Immunological aspects of aging. New York: Marcel Decker, 198 1.

Barrows, C. H., Kokkonen, G. C. Nutrition and aging: Human and animal laboratory studies. In: Ordy, J. M.; Harman, D.; Alfin-Slater, R., eds. Nutrition in gerontology. New York: Raven Press, 1984:279–322.

Barnard, D.; Knapka, J .; Renquist, D. The apparent reversal of a wasting syndrome by nutritional intervention in *Saquinus*. J. Lab. Anim. Sci. 38:42–56; 1988.

Benjamin, M. Outline of veterinary clinical pathology. 3d ed. Ames: IA: Iowa State University Press, 1978.

Borkan, G. A. ; Norris, A. H. Assessment of biological age using a profile of physical parameters. J. Gerontol. 35: 177-l 84; 1980.

Boume, G. H. Collected anatomical and physiological data from the rhesus monkey. In: Boume, G. H., ed. The rhesus monkey. vol 1. New York: Academic Press, 1975: l-38.

Cutler, R. G. Evolutionary biology of aging and longevity in mammalian species. In: Johnson, J. E., ed. Aging and cell function. New York: Academic Press, 1984: 1-147.

Gillibrand, D.; Grewal, D.; Blattler, D. P. Chemistry reference values as a function of age and sex, including pediatric and geriatric subjects. In: Dietz, A. A., ed. Aging — its chemistry. Washington, DC: The American Association for Clinical Chemistry, 1980:366–389.

Hanson, B. C.; Jen, K. C. Caloric intake and weight change in adult rhesus monkeys. In: Hayes, K. C., ed. Primates in nutritional research. New York: Academic Press, 1979:59–71.

Hutcheson, D. P.; Tumbleson, M. E.; Middleton, C. C. Serum electrolyte concentrations in Sinclair(S- 1) miniature swine from 1 through 36 months of age. Growth 43:62–70; 1979.

Jones, M. L. Longevity of primates in captivity. Internat. Zoo Yrbk. 8: 183–192; 1968.

Kessler, M. J.; Rawlins, R. G.; London, W. T. The hemogram, serum biochemistry, and electrolyte profile of aged Rhesus monkeys *(Macaca mulatta)*. J. Med. Primatol. 12:184–191; 1983.

McCay, C. M.; Crowell, M. F.; Maynard, L. A. The effect of retarded growth upon the length of the life span and upon the ultimate body size. J. Nutr. 10:63–79; 1935.

McClure, H. M. Hematologic, blood chemistry, and cerebrospinal fluid data for the rhesus monkey. In: Bourne, G. H., ed. The rhesus monkey. vol. 1. New York: Academic Press, 1975:409–427.

Masoro, E. J. Food restriction in rodents: An evaluation of its role in the study of aging. J. Gerontol. Biol. Sci. 43:B59–B64; 1988.

Morin, M. L.; Renquist, D. M.; Knapka, J.; Judge, F. J. The effect of dietary crude fiber levels on rhesus monkeys in quarantine. Lab. Anim. Sci. 28:405–411; 1978.

National Research Council. Committee on Animal Nutrition, Agricultural Board. Nutrient requirements of nonhuman primates. Washington. DC: Nat ional Academy of Sciences. 1978.

Pickering, R.; Pickering, C. E. The effects of reduced dietary intake upon the body and organ weights, and some clinical chemistry and haematological variates of the young Wistar rat. Toxicol. Lett. 2 1:27 1; 1984.

Portman, 0. W. Nutritional requirements of squirrel and wholly monkeys. In: Harris, R. S., ed. Feeding and nutrition of nonhuman primates. New York: Academic Press, 1970: 159-174.

Rao. G. N.; Knapka. J. J. Contaminant and nutrient concentrations of natural ingredient rat and mouse diets used in chemical toxicology studies. Fund. Appl. Toxicol. 9:239–338; 1987.

Renquist, D. M.; Judge, F. Use of nylon balls as behavioral modifiers for caged primates. Primate Newslet., October 1985.

Sachan, D. S.; Das, S. K. Alterations of NADPH-generating and drug-metabolizing enzymes by feed restriction in male rats. J. Nutr. 112:2301; 1982.

Short, R.; Williams, D. D.; Bowden, D. M. Cross-sectional evaluation of

potential biomarkers of aging in pig-tailed macaques: Effect of age, sex, and diet. J. Gerontol. 42:644–654; 1987.

Tumbleson, M. E.; Wingfield, W. E.; Johnson, H. D.; Campbell, J. R.; Middleton, C. C. Serum electrolyte concentrations, as a function of age, in female dairy cattle. Cornell Vet. 63:58–64; 1973.

Tumbleson, M. E.; Hutcheson, D. P.; Middleton, C. C. Serum protein concentrations and enzyme activities, as functions of age and sex, in Sinclair(S- 1) miniature swine. Growth 40:53–68; 1976.

Walford, R. L. The 120-year diet. New York: Simon and Schuster; 1986.

Webster, I. W .; Logie, A. R. A relationship between functional age and health status in female subjects. J. Gerontol. 3 1:546–550; 1976.

Weindruch. R.; Walford, R. L. The retardation of aging and disease by dietary restriction. Springfield. IL: Charles C Thomas, 1988.

Yoshida, T.; Suzuki, K.; Shimizu, T.; Cho, F.; Honjo, S. The effects of ketamine administration on hematological and serum biochemical values in female Cynomolgus monkeys *(Macaca fusciularis)*. Exp. Anim. 35:455–46 1; 1986.

Received March 10, 1989
Accepted November 2, 1989